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Single-channel study of the binding–gating coupling in the slowly desensitizing chimeric α7-5HT3A receptor

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ABSTRACT

We have studied the role of the highly conserved residue αLysine145 in the early steps of activation by acetylcholine of the nicotinic acetylcholine receptor (nAChR). Both macroscopic and single-channel currents were recorded in the slowly desensitizing chimeric mutant receptor α7V201-5HT3A/R432Q/R436D/R440A, made of α7 nAChRs and serotonin receptors of subtype 3A (ch1), and its corresponding mutant K145A (ch1/K145A) expressed in *Xenopus* oocytes. Mutant ch1/K145A receptors had a reduced gating function similar to that produced by the same mutation in the wild type receptor α7. The mutated receptor has reduced opening rate constants, *β*, and increased closing rate constants, α.

1. Introduction

Nicotinic acetylcholine receptors (nAChRs) mediate fast synaptic transmission in muscle and nerve cells. They are members of the Cys-loop family of ligand-gated ion channels, which includes 5-hydroxytryptamine3 (5-HT_3) receptors, gamma-aminobutyric acid (γ-$GABA_A$) receptors, and glycine receptors [1].

In a previous work [2] we determined the influence of the highly conserved residue Lysine145 in α7 receptors upon binding and gating by using measurements of macroscopic currents and binding of alpha-bungarotoxin (α-Bgt). The role of the same residue was also studied at the single-channel level in muscle receptors [3,4] where it was found that the mutation K145A produced a large reduction in the opening rate constant.

We also tried to study the role of K145 at the single-channel level in α7 receptors but the fast desensitization of the wild type receptor makes very difficult to obtain single-channel data, so we tried to use instead a mutated receptor slowly desensitizing and makes easier the task of obtaining long duration single-channel recordings.

In a recent work [5] we used the mutant α7L248T as our model receptor because it has very slow desensitization and it was possible to record single-channel currents in steady state conditions. Thus, we were able to study the effect of mutation K145A at the single-channel level. However, the mutant α7L248T was not a good model in other respects because it behaved differently than the wild type receptor when the pharmacology of the mutated receptor was studied. In particular, the mutation K145A in wild type α7 receptors produces a receptor that is inhibited by the agonist dimethylphenylpiperazinium (DMPP). On the contrary, the same mutation on α7L248T gives a receptor that is activated by DMPP, thus the mutation did not produce the alteration of pharmacology expected.

We decided to pursue further the research of the mutation K145A at the single-channel level by testing another construct that allowed us the recording of microscopic currents in stationary conditions. So we used the chimera receptor α7V201-5HT3A/R432Q/R436D/R440A as our model (referred as "ch1" hereafter). This chimera is constructed of α7 from the N-terminus up to the position Val201 and the rest is made of serotonine (5-HT_{3A}) receptor [6]. In addition, the three arginine mutations within the large cytoplasmic loop of the receptor result in a mutant channel with a conductance large enough to be recorded directly [7].

The recording of whole-cell macroscopic currents revealed that the mutation ch1/K145A produced a receptor with the same pharmacology that the original α7/K145A mutant receptor, thus in this respect ch1/K145A constitutes a better model to pursue the study

Abbreviations: nAChRs, nicotinic acetylcholine receptors; 5-HT_3, 5-hydroxytryptamine3; γ-$GABA_A$, gamma-aminobutyric acid; α-Bgt, alpha-bungarotoxin; DMPP, dimethylphenylpiperazinium; MLA, methyllycaconitine; DHβE, dihydro-β-erythroidin

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of the role of K145 on the yet unresolved problem of coupling binding to gating in neuronal nicotinic receptors.

2. Materials and methods

2.1. Generation of mutants of the bovine α7 subunit

The bovine α7 cDNA was cloned in a derivative of the pSP64T vector [8] containing part of the pBluescript polylinker. To generate the mutants we annealed single-stranded oligonucleotides with the desired sequences and proper single-strand ends that could be easily ligated to the ends generated through restriction enzymes digestion of the α7 and/or the 5-HT_{3A} receptor cDNAs. The restriction enzyme sites for cloning the annealed oligonucleotides were either present in the original cDNA sequences or introduced as silent mutations [9] by using mutated oligonucleotides and PCR (25 cycles at 94 °C for 10 s, 60 °C for 30 s and 72 °C for 45 s).

2.2. Oocyte expression

Capped mRNA was synthesized in vitro using SP6 RNA polymerase, the mMESSAGEmMACHINE kit (Ambion, Texas) and the pSP64T derivative mentioned above. Defoliculated *Xenopus laevis* oocytes were injected with 550 μg/μl of total cRNA in 50 nl of sterile water. Oocytes were incubated in calcium-free medium. All experiments were performed within 3–4 days after cRNA injection.

2.3. ^{125}I-α-bungarotoxin binding assays

Specific surface expression of ^{125}I-α-bungarotoxin (^{125}I -α-Bgt) binding sites was tested with 10 nM ^{125}I-α-Bgt as described [10]. Briefly, oocytes located in 24-well plates were preincubated for 15 min with Barth's buffer containing 5% of fetal calf serum and further incubated in the same medium with 10 nM ^{125}I-α-Bgt for 2 h at 18 °C in a final volume of 300 μl. At the end of the incubation, unbound ^{125}I-α-Bgt was removed, oocytes were passed to six-well plates, washed five times with 4 ml Barth's buffer and bound radioactivity was counted. Non-specific binding was determined using uninjected oocytes.

2.4. Macroscopic whole-cell recordings

Electrophysiological recordings of whole-cell currents were done as described previously [10]. The extracellular solution contained (in mM): NaCl 82.5, KCl 2.5, $BaCl_2$ 2.5, $MgCl_2$ 1 and HEPES 5 (pH 7.4). The velocity of application of agonists was 18–22 ml/min. All experiments were done at room temperature (22 °C). Unless otherwise indicated, oocyte membrane potential was held at −40 mV. Macroscopic currents were measured with the Clampfit 9 (Molecular Devices) software. Data points are shown as means with standard errors. Data analysis was performed with Prism 4.0 (GraphPad Software Inc., San Diego, CA, USA). Current–concentration curves were fitted using a non-linear least squares algorithm to the Hill equation, $I/I_{max} = 1/(1 + (EC_{50}/C)^n)$, were EC_{50} is the agonist concentration that elicits the half-maximal response, n is the Hill coefficient, and C is the agonist concentration. Affinity constants were calculated by a modified Cheng–Prusoff equation [11] .

2.5. Single-channel recordings

Single-channel records were obtained in the cell-attached mode using an Axopatch 200A amplifier (Axon Instruments, CA). Oocytes were bathed in a modified extracellular solution where NaCl was substituted by KCl to clamp the membrane potential close to 0 mV. Holding potential of the patch was −100 mV. Patch pipettes were pulled from thick-walled borosilicate glass (GC150-15, Clark Electromedical Instruments), with a resistance between 5 and 10 MΩ when filled with standard extracellular solution. ACh or DMPP were added to the pipette solution. Records were low-pass filtered at 1 kHz with an 8-pole Bessel filter (Frequency Devices Inc.), sampled at 20 kHz and stored on hard disk for later analysis.

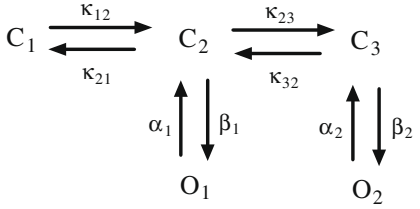
Single-channel recordings were analyzed with the Clampfit 9 and the QUB-1.4.0.2 software suite [12]. For each patch a voltage pulse protocol was applied to determine the conductance (γ) and reversal potential (V_r) of the channels. Ten second duration pulses were applied from +60 to −120 mV at 20 mV intervals. Single-channel current–voltage curves were fitted to the equation $I = \gamma(V - V_r)$. Open probabilities were estimated from all point histograms fitted to a sum of Gaussians.

Kinetic analysis was performed on records with a dead time of 0.1 ms, idealized by the half amplitude threshold method, and restricted to bursts of activity of a single-channel. Bursts were identified by a closed interval longer than a given critical time that usually was between 30 and 100 ms, and containing more than three opening events. The selected region of the idealized data was then fitted to a simple kinetic model with three closed and two open states, with eight kinetic constants, as shown in kinetic Scheme 1, with the MIL method that gives directly an estimate of the kinetic constants and their standard errors. This scheme has been used here because it has the minimum number of states needed to fit the open and closed dwell time histograms. Dwell time histograms with the corresponding time constants, and their relative weight, were also obtained with the same program. Statistical analysis of the fitted parameters and t-tests for the significance of the difference between two sample means were performed with SPSS14 (SPSS Inc.).

3. Results

3.1. Effect of the mutation K145A on the functional responses to ACh in the slowly desensitizing chimera receptor ch1

Fig. 1A shows two families of macroscopic inward ACh-evoked currents in chimeric ch1 and mutant ch1/K145A receptors, respectively. Inward currents evoked at equivalent ACh concentrations had the same kinetics both in ch1 and mutant ch1/K145A receptors. In average, maximal currents in mutant ch1/K145A were around 9-fold smaller than in control despite the fact that the electromotive force was 2-fold larger, and surface expression, as measured by α-Bgt binding, was similar in both cases (ch1 and mutant oocytes expressed 31 ± 3 and 35 ± 3 fmol/oocyte of α-Bgt, respectively). Thus, after normalizing functional responses to surface expression and taking into account the difference in electromotive force, mutant ch1/K145A showed a gating function (current to binding ratio) of approximately 8% of control. This reduction in gating is larger than that obtained after the inclusion of the same mutation in wild type α7 receptors [2]. On the other hand, as shown in Fig. 1B, mutant ch1/K145A had a decrease in ACh potency



Scheme 1.

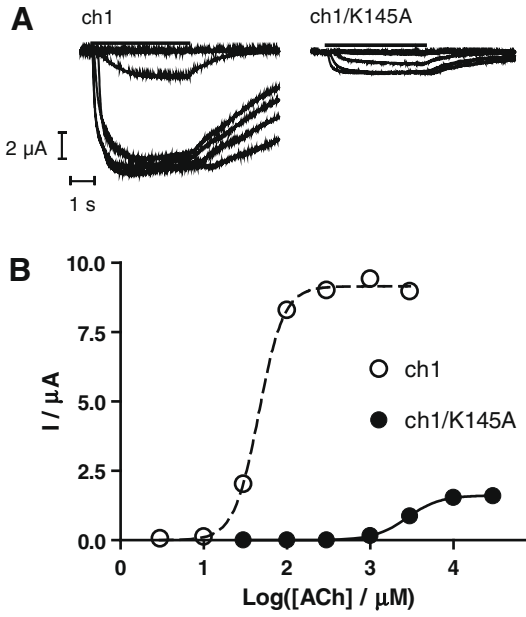


Fig. 1. A comparison between the ACh responses of chimera ch1 and mutant ch1/K145A. (A) Inward currents obtained upon stimulation with ACh of ch1 and ch1/K145A mutants. Traces represent currents obtained at −40 mV with ACh concentrations from 3 to 3000 μM in ch1 and at −80 mV with ACh concentrations from 30 up to 30000 μM for the mutant ch1/K145A. The bar over the traces indicates the period of 4 s during which the ACh was applied. (B) ACh maximal current–concentration curves of single oocytes expressing ch1 receptors (open circles) and K145A mutants (solid circles) for the maximal currents shown in (A). Lines represent fits to the Hill equation. ch1: I_{max} = 9.2 ± 0.1 μA, EC_{50} = 46 ± 2 μM and nH = 2.9 ± 0.2. Mutant ch1/K145A: I_{max} = 1.62 ± 0.01 μA, EC_{50} = 2780 ± 35 μM, n = 2.2 ± 0.1. These particular oocytes were chosen because the results of the individual fit parameters were close to the average values (Table 1 in Supplementary data), although their desensitizing kinetics is slower than average.

of 47-fold, similar to that reported for mutant α7/K145A receptors. As occurred in the mutation made on α7 wild type receptors, this change in the EC_{50} value is not quantitatively predicted by considering only the modification in gating [13]. Thus, a change in the ACh binding properties is likely to have taken place as well.

3.2. Pharmacological profile

Mutations of Lys145 in α7 wild type receptors induced changes in the pharmacological profile of the resultant receptors [2]. Therefore, we checked the functional responses of ch1 and mutant ch1/K145A receptors to several nicotinic drugs. All agonists tested evoked ionic currents that were kinetically indistinguishable from ACh-evoked currents both in ch1 and in mutant ch1/K145A receptors (not shown).

Fig. 2 shows representative concentration-current relationships for carbamylcholine, cytisine, nicotine and epibatidine. When compared to ch1 curves, those obtained with mutant ch1/K145A were consistently shifted to the right, showing statistically significant higher EC_{50} values, and all, except epibatidine, showed a large reduction in the maximal current (note that ch1 currents in Fig. 2 were measured at −40 mV while mutant currents obtained with CCh, Cyt and Nic were measured at −80 mV because of their smaller size).

We did the same analysis for each individual oocyte, selecting only those for which it was possible to obtain the whole current–concentration curve, and averaged the resulting fitting parameters. The results are summarized in Table 1 in supplementary data. The differences in EC_{50} were statistically significant for all the agonists tested. The main differences in EC_{50} were observed in ACh, cytisine and nicotine where the mutation produced a shift of approximately 50-, 40- and 30-fold, respectively, whereas the shift was only 7- and 6-fold for carbamylcholine and epibatidine, respec-

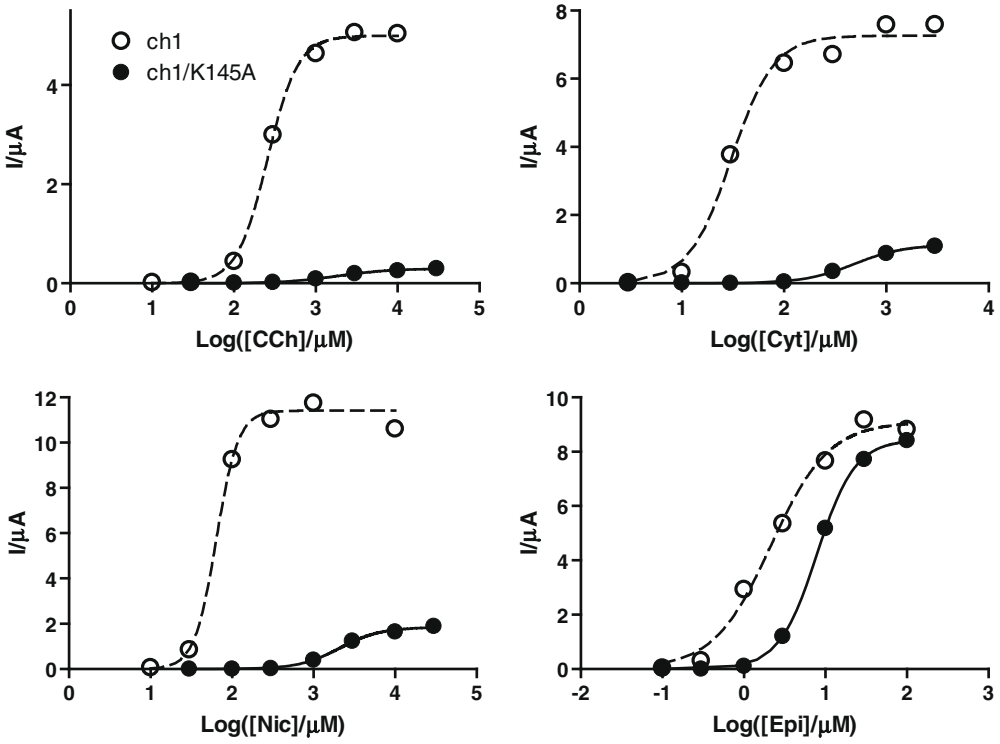


Fig. 2. Pharmacological profile: agonists. Current–concentration curves obtained for ch1 (open circles) and mutant receptors (filled circles) for four different agonists: carbamylcholine (CCh), cytosine (Cyt), nicotine (Nic) and epibatidine (Epi). Lines represent fits of data to the Hill equation with the following parameters (I_{max}/μA, EC_{50}/μM, n): CCh ch1 (5.0, 260, 2.3), CCh mutant (0.3, 1890, 1.3); Cyt ch1 (7.3, 30, 2.1), Cyt mutant (1.1, 490, 1.8); Nic ch1 (11.4, 64, 3.3), Nic mutant (1.8, 2090, 1.7); Epi ch1 (9.1, 2.2, 1.2), and Epi mutant (8.4, 7.9, 1.9). All currents were measured at −40 mV, except for the mutants with CCh, Cyt and Nic that were measured at −80 mV because of their smaller size.

tively. On the other hand, the differences in maximal currents were also significant, except for epibatidine, and their magnitude was not correlated with the shift in EC_{50}. Thus, the reductions in I_{max} were small (about 5-fold) for ACh, nicotine and cytisine, and was greater for carbamylcholine (almost 25-fold).

The large reduction in I_{max} for carbamylcholine was not as great as reported in α7/K145 where this agonist became a competitive antagonist. However, as happened with α7/K145A, that change from agonist to antagonist was in fact observed here for ch1/K145A with DMPP. In ch1 receptors, DMPP evoked currents similar to the other agonists; its concentration-current curve was very similar to that of cytisine ant its potency was only inferior to that of epibatidine. In contrast, we were unable to detect any currents when DMPP was applied to ch1/K145A receptors. The effect of DMPP on ch1/K145A receptors was similar to other competitive nicotinic antagonists as methyllycaconitine (MLA), as shown in Fig. 3. In ch1/K145A mutant receptors, DMPP was able to inhibit the currents elicited by 10 mM ACh with an IC_{50} of 80 μM approximately, a value almost 30 times larger than that of MLA. For nicotinic antagonist MLA the mutation ch1/K145A gave as a result a significant shift in IC_{50} towards smaller concentrations by approximately a factor of 4. The corresponding values of the affinity constant for the inhibition, K_i, were: 35 μM for DMPP on ch1/K145A and only 0.8 μM and 1.2 μM for MLA on ch1 and ch1/K145A, respectively. The difference between these last two values was not statistically significant. Similar results were obtained for dihydro-β-erythroidin (DHβE), data not shown.

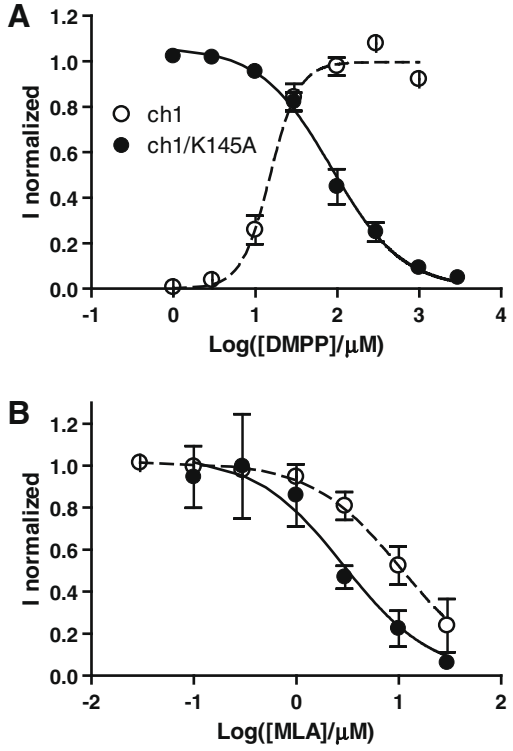


Fig. 3. Pharmacological profile: antagonists. Normalized activation and inhibition curves. For each individual oocyte activation or inhibition data (i.e. current–concentration curves) were fitted to the Hill equation and the resulting I_{max} was used to normalize the values of the current. The data points in the figures show the averaged values and standard errors of the normalized values. The lines are fits to the Hill equation of the resulting averages for ch1 (open circles, dotted lines) and the mutant ch1/K145A (solid circles and lines), for DMPP (panel A) and for the antagonist methyllycaconitine (MLA) (panel B). Inhibition curves were obtained by co-applying the different antagonist concentrations with 1 mM ACh for ch1 or 10 mM ACh for ch1/K145A with a Hill coefficient of −1. Fit parameters are summarized in Table 1 in supplementary data.

3.3. Unitary currents in ch1 and ch1/K145A

So far as macroscopic currents are concerned, substitution of Lys145 by alanine showed the same effects in ch1 than in wild type α7 receptors. However, we could study the role of Lys145 in more detail in ch1 than in wild type α7 receptors because the slowly desensitizing properties of ch1 allowed acquiring single-channel recordings in the cell-attached configuration of the patch-clamp technique.

Fig. 4 shows typical cell-attached single-channel recordings at different concentrations of ACh in ch1 and ch1/K145A receptors. For each receptor, three different concentrations are shown. Those concentrations have been chosen, according to the macroscopic current–concentration curves, corresponding to the steep part of the curve and are roughly equipotent for each receptor.

In both receptors the open probability was higher in those patches with higher ACh concentration. This can be seen from the relative area of the second peak in the all points histograms, that correspond to the openings of the channel. The overall open probability was small as can be inferred by the large relative magnitude of the baseline peak compared with the second peak. In the figure, the largest open probability corresponds to the patch with ch1 receptors with ACh 100 μM, and it was approximately 10%.

As can be seen in Fig. 4, the magnitude of the currents at −100 mV was usually larger in ch1 (about 7.5 pA) than in ch1/K145A (5 pA approximately), this difference is probably a consequence of agonist block as the conductance of the channel decreased with increasing concentration, although not in a linear manner (see bottom of Fig. 4). The single-channel conductance measured between −20 and −120 mV with different ACh concentrations ranged between 86 ± 5 pS at 10 μM, and 50 ± 2 pS at 10 mM for ch1; and between 77 ± 13 pS at 3 mM, and 16 ± 3 pS at 30 mM for ch1/K145A. No different conductance levels were observed in a given patch. The extrapolated value for the reversal potential was close to 0 mV for both receptors.

In Fig. 4, the duration of the openings was also larger in ch1 than in ch1/K145A at the corresponding concentration, and a more detailed analysis is presented in the following section.

3.4. Single-channel kinetics in ch1 and ch1/K145A

Mutant ch1/K145A has shorter openings than ch1. Fig. 5 shows typical records of 200 ms duration with openings for ch1 and ch1/K145A for four different agonists. The agonist concentrations used were 100 μM for ch1 and 1 mM for ch1/K145A in all cases except for epibatidine in which 10 μM was used for both receptors. The data in recordings of 5 min were idealized by the half amplitude method and divided into segments with clusters of activity defined by having a closed time longer than a given burst terminator time, between 30 and 100 ms depending on the activity of the channel. Bursts with more than one open level, indicating the activity of multiple channels, were discarded. The resulting dwell time histograms are shown in Fig. 5 at the right of the records. The histograms were fitted to the kinetic model given in the methods section and the averaged values of the fitted parameters for different number of patches are shown in Table 2 in supplementary data.

For the time constants the data show that comparing a given receptor with different agonists, and taking ACh as our reference, the only statistically significant differences were for the longer open time constant (τ_{o2}): with Cytisine it was four times smaller, whereas with Epi it was approximately three times larger. On the other hand when the dwell times for ch1 were compared with those for ch1/K145A, for each agonist, the statistically significant differences indicated that the mutant receptor had usually longer closed times and especially that the duration of the longer openings was reduced by approximately 5-fold. These results suggest

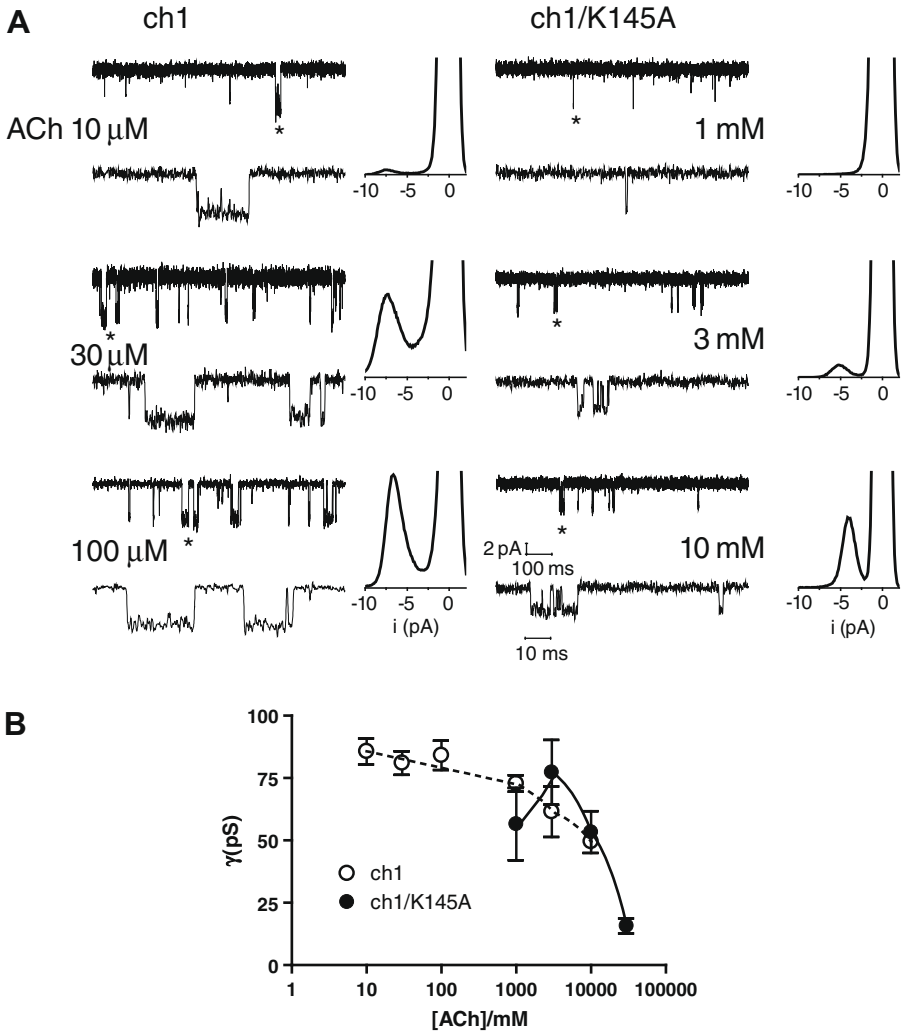


Fig. 4. Single-channel recordings with ACh. (A) Representative traces of single-channel currents evoked by ACh in ch1 (left) and mutant ch1/K145A receptors (right) at different concentrations. Openings are downward. For each agonist concentration, a record of 1 s duration is shown. The region of each record marked with an asterisk is shown below at a 10-fold expanded time resolution. The histograms at the right of each record are all points histograms created from 5 min long recordings, normalized to have a total area of one. The scale is the same in all histograms, and the first peak (out of scale) corresponds to the baseline level. (B) Single-channel conductance as a function of agonist concentration for ch1 (open dots) and ch1/K145A mutant receptors (solid dots).

that the effect of mutation K145A is both impairment in the channel activation and destabilization of the open configuration.

In terms of the kinetic constants of the model used to analyze the data, the main differences seen with the mutant ch1/K145A that are reproduced for all agonists were with the kinetic constants β_2 and α_2. The opening rate constant β_2 is reduced in the mutant by a factor between 3 (in the case of ACh) and 10 (for epibatidine). On the other hand, the closing rate constant α_2, that determines the mean dwell time of the open state with longer duration, is increased by the mutation by a factor of between 2 and 10 (for epibatine and nicotine, respectively), thus making the transition from the open to the closed state faster in that factor. Both changes suggest again that in ch1 receptors the mutation K145A impairs the channel activation and destabilizes the configuration of one of the open states.

4. Discussion

The mutation K145A in chimeric receptors ch1 had very similar effects to those observed in wild type α7 receptors at macroscopic level [2], i.e. the maximal current obtained with ACh decreased and the current–concentration curve was shifted towards higher concentrations by almost two orders of magnitude. Such large shift cannot be quantitatively explained by a simple reduction in gating [13]. The number of receptors present in the surface of the oocytes, as determined by α-Bgt binding experiments was not affected by the mutation whereas a small reduction of about 25% was observed when the same mutation was introduced in α7 receptors.

The pharmacological profile of the mutated ch1/K145A receptors was also very similar to its counterpart in α7 receptor. Lys145 affects the effectiveness of ACh, CCh, nicotine, cytisine and DMPP. The main effect being the conversion of DMPP from agonist to competitive antagonist. Moreover, as in α7 receptors, epibatidine was the less affected agonist. Competitive nicotinic antagonists also seemed to change their properties in mutant ch1/K145A receptors as revealed by the shifts in the inhibition curves of the α7-selective antagonist MLA. Again this effect is similar to the one observed in mutated α7 receptors, however, the quantitative details are different. In α7 the mutation produces an increase in the potency of the antagonist, as revealed by a decrease in the affinity constant K_i by a factor of 6, whereas in ch1 mutants the change in K_i was not significant. The contributions of residues of the extracellular domain to the pharmacological profile of the nicotinic receptor has been reported previously [14–17] and thus

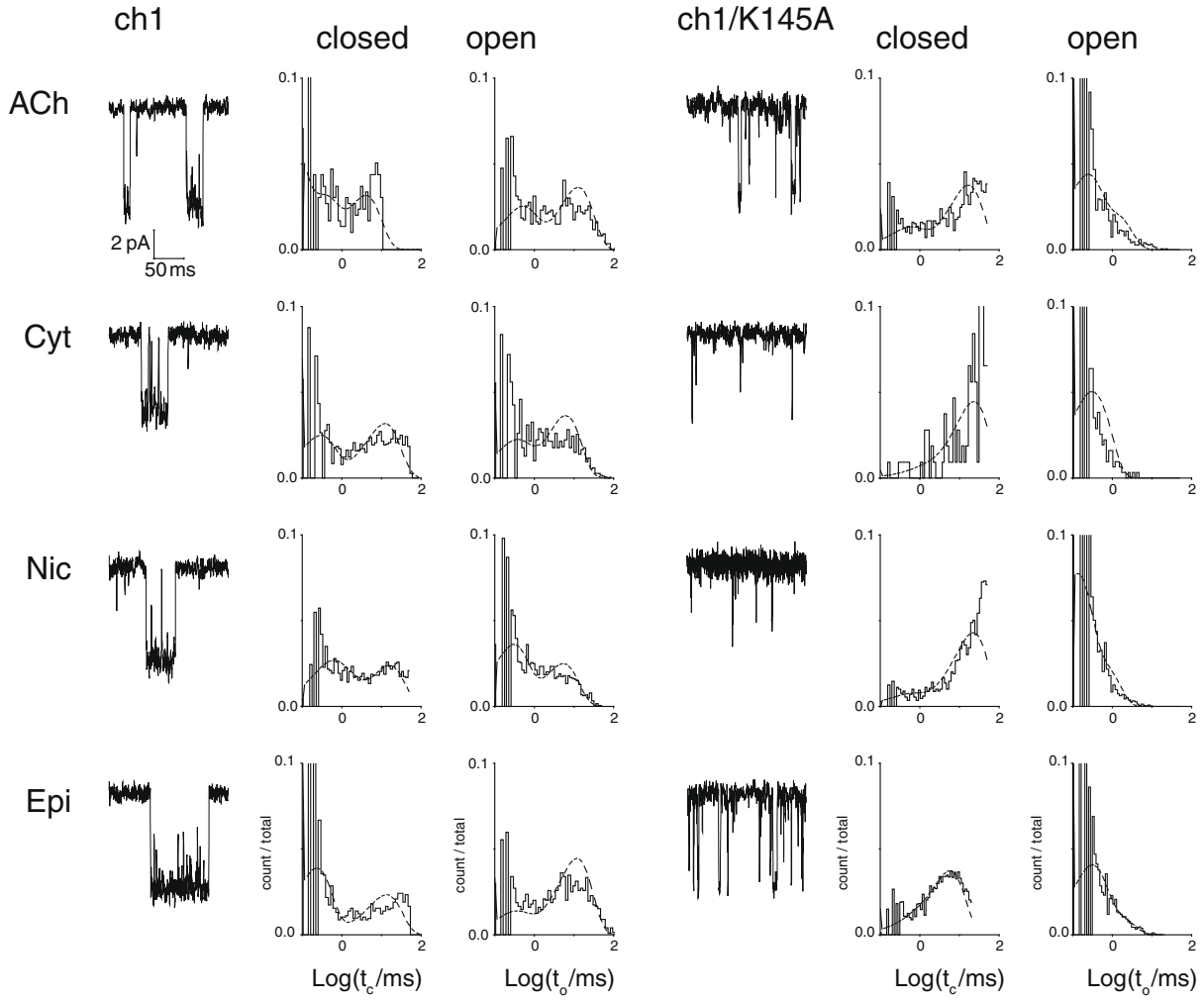


Fig. 5. Dwell time histograms of single-channel events. Sample single-channel recordings with openings and histograms for closed (t_c), and open (t_o) time for ch1 (left) and mutant ch1/K145A (right) receptors with different agonists: acetylcholine, cytisine, nicotine and epibatidine. All the records are of 200 ms duration and shown at the same scale. The dwell time histograms were obtained from records of 5 min duration. Logarithmic binning and linear scale for relative frequency of events were used. The analysis was restricted to bursts of activity usually limited by closed times shorter than 100 ms, or 30 ms in cases of patches with more than one channel and large activity. Superimposed to the histograms are the fits, in dotted lines, to kinetic Scheme 1.

our results confirm that residue K145 also plays a role in the determination of the pharmacological profile of nicotinic receptors.

The experimental model ch1 was used not only because it reproduced the effect of mutations on residue Lys145 on the macroscopic currents and pharmacological profile of wild type α7 receptors but also because it allowed us to study the function of the receptor at the single-channel level as the mutation of the arginines in the intracellular domain result in a channel with an increased conductance that makes it easy to measure [7]. Although we could not obtain stable outside-out patches to perform concentration jump experiments with agonists in order to investigate the microscopic mechanism of the channel function, we were able to obtain long duration recordings in the cell-attached configuration and the observed activity of the channel increased with ACh concentration in both ch1 and ch1/K145A receptors. The measured single-channel conductance of the mutated receptor was smaller than that of ch1, probably due to the fact that large concentrations of agonist have a blocking effect on the pore as reported [18]. This reduction in single-channel conductance, however, is not enough to account quantitatively for the reduction in function observed in the mutated receptors, thus a change in the kinetics of the receptor was also expected.

The kinetic analysis of single-channel recordings was complicated by the fact that many of the patches that we recorded had more than one channel present, so that closed times could not be attributed to a single receptor, instead we limited our analysis to bursts of activity with no overlapping openings. The activity of the patches was usually smaller in the mutated receptors so that bursts could be delimited by longer closed times. The longer closed times we used were 100 ms but in some patches with ch1 receptors and ACh we had to reduce this value to 30 ms due to a much larger activity. This change, however, did not produce statistical outliers when the results were analyzed for a given pair of receptor and agonist.

For agonists tested the mutated receptor had shorter open times and usually longer closed times than ch1 receptor. As previously reported [18], we also observed an open time distribution with two distinct time constants and both were reduced in the mutant receptor, but mainly the longer one (despite the fact that the higher concentrations used for the mutant can produce open channel block and therefore lengthen the open interval durations [19]), thus indicating that there is an increase in the values of the kinetic constants α_1 and α_2 that close the channel. There was also a change in the closed time distributions but the corresponding kinetic constant affected cannot be attributed to a single property of the closed dwell time distribution.

These results are in qualitative agreement with those obtained in muscle nAChRs showing that substitution of Ala for Lys145 in-

tensely affected receptor activation but mostly by a large reduction in opening rate constant [3,4], and also in neuronal nicotinic receptors as observed in our recent study on bovine α7 mutant receptors K145A-L248T that have impaired gating as a consequence of a slower opening rate constant [5]. Thus, our data also suggest that Lys145 is involved in the early steps of nAChR activation, and this would be expected given its location in the structure of the receptor [20], but also that Lys145 may play a role in stabilizing the open configuration of the channel. Quantitative discrepancies between muscle- and neuronal type nAChR gating have been reported previously [2,9,21,22].

Although we have used a chimeric receptor for studying the role of K145 in the gating mechanism of nicotinic receptors we think that our results can be extrapolated to the wild type receptor as we have shown that the pharmacological profile of the receptor is conserved and that the macroscopic effects of the mutation Lys145A are the same. The possibility of obtaining long duration single-channel recordings has enabled us to study the mechanism in more detail and to show that Lys145 is involved in the process of channel activation and stabilization of the open configuration of the neuronal nicotinic receptors.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2009.02.026.

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